whichever enzyme was used. It is most unlikely that the reductions of $1\alpha,2\alpha$ -epoxy- 17β -hydroxy- 5α -androstan-3-one involved any change other than reduction of the 3-oxo group. The $1\alpha,2\alpha$ -methylene compound was an inhibitor. The $1\alpha,2\alpha$ -epoxy and $1\alpha,2\alpha$ -methylene compounds may be expected to be closely similar in shape (ring A half boat), though both probably differ somewhat from the shape of the 1α -methyl and 2α -methyl compounds (ring A chair).

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The Effect of Thiols on Oestradiol Binding by Components of Human Malignant Breast Tumours

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The method of Feherty *et al.* (1971) has been used to study the effect of β -mercaptoethanol, dithiothreitol and L-cysteine on the concentration of oestrogen-binding sites in supernatant preparations (800g) from human breast tumours. Eight out of 12 tumours with measurable concentrations of binding sites (0.5-10.8 fmol/mg wet wt.) showed increased binding (1.2-fold to more than 6-fold) after treatment of the supernatant with mercaptoethanol (1-100 mM) for 10 min at 0°C. In only one out of 14 preparations that did not bind initially could binding be induced by mercaptoethanol. Pretreatment with dithiothreitol (0.1-10 mM) appears to have a greater effect (on an equimolar basis) than mercaptoethanol. In contrast, L-cysteine can decrease the ion-concentration of binding sites and increase K_d . Pretreatment of supernatants with a thiol-blocking agent (1 mM-N-ethylmaleimide) abolished subsequent binding of oestradiol as estimated by this technique.

These results confirm the involvement of thiol

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groups in oestradiol binding by components of human breast tumours (Hähnel & Twaddle, 1971). The enhancement of oestradiol-binding activity by some thiols may be associated with reduction of disulphide bridges or protection of thiol groups in the high-affinity binding component(s). The effect of L-cysteine may be competitive and may or may not involve its thiol group.

In some organisms the onset of cell division may be controlled by specific 'division' proteins and sulphur-containing macromolecules (Morris, 1965). The evidence for the involvement of thiol groups in growth has been reviewed by Harington (1967), and it is noteworthy that such groups appear to be involved in the binding of oestradiol by breast cancer preparations. However, Harington (1967) points out the difficulty of assigning significance to interaction with so highly reactive a group. Whether oestrogen binding is related to breast-tumour growth in human subjects is not known at the present time.

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Functional Groups in the Catalysis and Regulation of *Escherichia coli* Citrate Synthase

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The key position of citrate synthase in the tricarboxylic acid cycle favours it as a site for metabolic regulation, and allosteric feedback inhibition by both NADH and α -oxoglutarate has been demonstrated (Weitzman, 1966a,b; Wright *et al.*, 1967). We have subjected the purified enzyme to photo-oxidation and specific chemical modification in order to identify amino acids participating in the catalytic and regulatory processes.

Photo-oxidation with Methylene Blue demonstrates the involvement of three photo-oxidizable amino acids in catalytic activity and two in regulation (one in NADH inhibition and one in α -oxoglutarate inhibition). The dependences of the rates of photo-oxidation on pH, the characteristics of photo-oxidation with Rose Bengal and the results of amino acid analyses suggest that the susceptible residues involved in activity are two of histidine and one of methionine or tryptophan, and that those associated with regulation are cysteine (NADH inhibition) and histidine (α -oxoglutarate inhibition).

Supporting evidence is derived from specific modi-

fication studies, as follows. (a) Enzyme activity was destroyed by treatment with diethyl pyrocarbonate at pH 6.0, implicating histidine in activity. Although the inhibition by α -oxoglutarate was unaffected by this reagent, the pH-dependence of α -oxoglutarate inhibition further suggests the involvement of histidine in this regulation. (b) Treatment with 2-hydroxy-5-nitrobenzyl bromide at pH 4.6 caused a rapid loss of enzyme activity with no alteration of NADH or α -oxoglutarate inhibition. When one tryptophan residue/enzyme subunit was attacked by the reagent 10% of the initial activity remained. Modification of methionine by H_2O_2 at pH 6.0 caused no loss of enzyme activity or regulation. Together with the results of 2-hydroxy-5-nitrobenzyl bromide treatment this suggests that the third photo-oxidizable amino acid implicated in catalytic activity is tryptophan rather than methionine. (c) Modification of the enzyme with the thiol-specific reagent 5,5'-dithiobis-(2-nitrobenzoate) resulted in complete loss of NADH inhibition and, more slowly, of enzyme activity, but no loss of α -oxoglutarate inhibition. This confirms that cysteine is essential for NADH regulation and activity. The resistance of the active-site cysteine residue(s) to photo-oxidation may result from a quenching effect exerted by the photo-oxidation of other neighbouring residues.

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The Regulation of *Acinetobacter* sp. α -Oxoglutarate Dehydrogenase Complex

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Preliminary investigations on unpurified preparations of the α -oxoglutarate dehydrogenase complex from *Acinetobacter* sp. revealed the following regulatory properties (Weitzman, 1972). (a) AMP and ADP stimulate enzyme activity by lowering the K_m for α -oxoglutarate, whereas ATP is slightly inhibitory; (b) NADH inhibits the complex; (c) AMP and ADP overcome the NADH inhibition.

The complex has now been purified to homogeneity as indicated by gel electrophoresis and sedimentation-velocity analysis. The purified α -oxoglutarate dehydrogenase complex responds to NADH and adenylates as above. We have attempted to identify the effector sites of action by means of assays specific for the component enzymes.

The first enzyme component, E1 (α -oxoglutarate

dehydrogenase), has been assayed by electron transfer to 2,6-dichlorophenol-indophenol by following the reaction at 578 nm. The effects of AMP and ADP on component E1 were similar to those observed for the whole complex. The effect of NADH on component E1 could not be tested in this way owing to the non-enzymic reduction of 2,6-dichlorophenol-indophenol.

The third enzyme component, E3 (lipoamide dehydrogenase), has been assayed spectrophotometrically and polarographically. NADH was a competitive inhibitor with respect to NAD^+ , whereas the adenine nucleotides were without effect on enzyme activity and on the NADH inhibition of component E3. This result contrasts with the relief of NADH inhibition by AMP and ADP observed with the whole complex, suggesting an additional site of action for NADH.

Evidence to support this suggestion has been obtained from kinetic studies: (a) the inhibition of the complex by NADH is competitive with respect to α -oxoglutarate; (b) although NADH inhibition is competitive with respect to NAD^+ it is not overcome completely at high NAD^+ concentrations; (c) detailed kinetic analysis indicates two binding sites for NADH. These results are consistent with an NADH-inhibitor site on component E1.

Studies on the adenylate regulation of the complex have shown that the effectors act allosterically. Treatment with 1 M-urea desensitizes the complex to AMP and ADP without loss of activity. This desensitization is accompanied by a lowering of the K_m for α -oxoglutarate.

The effects of adenylates and NADH on the α -oxoglutarate dehydrogenase complex are consistent with a regulatory role within the tricarboxylic acid cycle. As might be expected, these controls operate on the first enzyme component and the NADH inhibition represents a feedback mechanism within the complex itself.

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A Simple Method for the Determination of the Endo or Exo Nature of Carbohydrases Acting on Polymeric Substrates

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Several methods are known for distinguishing between the endo and exo nature of some carbohydrases acting on their polymeric substrates (Nelson